POSTER PRESENTATION

Flea'in Around: A Look at the Identification, Preservation, Clearing, and Mounting of Siphonaptera

James D. Campbell, **Steve Bennett**, **Laura Krueger**, **Tim Morgan**, **Kiet Nguyen**, **Amanda Penicks**, **Sokanary Sun**, and **Robert Cummings**
Orange County Mosquito and Vector Control District, Garden Grove, California
Danielle Martinez
Biological Sciences, College of Natural Sciences and Mathematics, California State University, Fullerton, California
Niamh Quinn
University of California Cooperative Extension, Irvine, California

ABSTRACT: Fleas are remarkable and highly specialized insects, with no part of their external anatomy being easily mistaken for that of any other insect. Due to their small size, the subtle differences among the distinguishing morphological characteristics of each species, and complexities of preparing specimens, identifying, and working with fleas is challenging. Various documents and taxonomic keys are available that discuss mounting procedures and the identification of medically important fleas for large regions of the world including the United States; however, many of these have become antiquated over time. Some of the distinguishing specialized characteristics exhibited among flea species, as presented in older keys, come in the form of line drawings, which are accurate but can be difficult to use when comparing it to structures on a whole specimen when viewed through a microscope. This paper presents a guide which describes in detail previously developed, but obscure techniques covering the preservation, preparation, clearing, and mounting of Siphonaptera specimens. In addition, we are also presenting an easy-to-use photographic key of twelve flea species collected from back yard wildlife, as well as pet cats and dogs in Orange County, CA. This key, which is freely available online at the Orange County Mosquito and Vector Control District's website, is an effective tool for the identification of common flea species found in southern California. Using the key in conjunction with the mounting guide will provide users with a full-circle guide to preserving, identifying, and mounting flea specimens. Keyed flea genera include *Cediopsylla*, *Ctenocephalides*, *Diamanus*, *Echidinophaga*, *Hoplopsyllus*, *Leptopsylla*, *Nosopsyllus*, *Orchopoeas*, *Pulex*, and *Xenopsylla*. Examined hosts include cats, coyotes, dogs, mice, opossums, rabbits, raccoons, rats, skunks, squirrels, and woodrats.

KEY WORDS: California, *Ctenocephalides*, *Diamanus*, *Echidnophaga*, flea, identification, mounting guide, pictorial key, *Pulex*, Pulicidae, Siphonaptera, *Xenopsylla*

Proc. 28th Vertebr. Pest Conf. (D. M. Woods, Ed.)
Published at Univ. of Calif., Davis. 2018. Pp. 329-333.

INTRODUCTION

Fleas are highly specialized holometabolous arthropods, with both males and females consuming blood for sustenance. These creatures are ectoparasites on a wide range of mammals and birds. Every seta, comb, bristle, and morphological development, such as a laterally compressed body, is adapted for life within the fur, hair, or feathers of their host. This means that their presence on a host's pelage can be very cryptic and might go unnoticed by those not looking for them. Due to their close relationship with their host and need to metabolize blood for sustenance, fleas are key vectors of pathogens causing such diseases as the plague (*Yersinia pestis*), where their role in transmitting this disease agent has been documented since the late 1800s. Being of medical importance it is essential to keep individuals educated and trained on the systematics and taxonomy of fleas, especially within the public health realm. With all the unique features present in the anatomy of fleas, along with the new and odd terminology that comes with studying these insects, identifying or simply trying to examine the anatomy of species from the order Siphonaptera can be interesting and rewarding, yet very difficult for those new to the study of fleas.

A Look at the Cranium

The head of the flea is an undoubtedly specialized capsule, with many characteristics that are distinct to the order Siphonaptera (Snodgrass 1946). The cranium of the flea is positioned exceptionally close to the notum of the thorax, sometimes protruding into it. The neck and pronotum are covered by a head flange, an exoskeletal plate protruding off the back of the cranium. This overlap will often make the first pair of legs appear to be originating from the head, and not the thorax. The surface of the head is devoid of any grooves or sulci (sometimes referred to as "sutures") that normally demark common regions/areas of an insect's head (Snodgrass 1946).

The two most noticeable features of the cranium are the presence of ctenidia, and antennal grooves. A ctenidium, commonly referred to as a "comb," is a row of strong spines located on the head of the flea. It is typical to find fleas with a variety of comb arrangements ranging from 1) completely lacking any combs, to 2) possessing one comb, to 3) possessing two combs, referred to as the genal and pronotal combs. The genal comb of a flea can be commonly confused for the teeth or mouth of the insect by individuals new to the study of Siphonaptera, due to its placement at the front of the head, where one

would typically find mandibles in other arthropods. These combs assist the flea in resisting any back pulling that commonly occurs when a host tries to scratch or remove the flea from its fur/hair. Antennal grooves, termed antennal fossae, can be found on either side of the head just behind the back of the eye (the anterior wall of the fossa is fused to the sclerotic cup of the eye), and house the individual antenna of the flea (Snodgrass 1946). The fossae happen to be large enough that they form deep incisions into the lateral walls of the head, causing the head cavity to be restricted between the fossae (Snodgrass 1946). On some fleas the walls of the fossae protrude far enough into the head that they touch, and in other species the inner wall of each fossa is untied/fused with one another (Snodgrass 1946). Fleas rarely lift their antenna out of the grooves; females almost never raise them up, and males only do so during copulation, when they are used to help hold the female over the male's back.

A Look at the Feeding Apparatus

The feeding apparatus of the flea is a specialized piercing and sucking structure, with features again distinct to Siphonaptera. The pharynx of the flea is located within the cranium just above the eye. The opening of the pharynx is considered the true "mouth" of the flea, and butts up against a preoral cibarial pump, which helps pull the bloodmeal through the epipharynx (Snodgrass 1946). Leading down from the cibarial pump is the epipharynx, the canal or path the bloodmeal uses to get to the cibarial pump. The epipharynx, which is solely unique to Siphonaptera, runs down from the end of the cibarial pump to the base of the epipharyngeal stylet, and shares a common chamber with the hypopharynx, where both bloodmeals and saliva pass through to get to their respective destinations (Snodgrass 1946). The hypopharynx, which can be difficult to distinguish due to its small size, has a readily viewable exposed portion which is a very small, flat, and heavily decurved projection, and is located close beneath the base of the epipharynx, and inserts into the food canal between the recurved base of each maxillary stylets (Snodgrass 1946). The hypopharynx contains the exit canal for the salivary pump, and its positioning suggests that saliva is dropped onto the basal end of the hypopharynx and then enters the food canal via canals or grooves on each maxillary stylet.

The exposed portion of the feeding apparatus, which is injected into the host, consists of three tendril-like stylets. There is a pair of maxillary stylets, termed the maxillary lacinea, which are long blade like structures that are convex on their outer surface, concave on their inner surface, and possess a strong midrib that runs the entire length of the stylets (Snodgrass 1946). The distal end of each stylet is armed with four rows of denticles (teeth-like structures), and the end of each stylet/blade is blunt and exhibits several small projections. Observations made on the feeding habits of the cat flea, *Ctenocephalides felis*, shows that this pair of stylets is used to break or cut the surface of the host's epidermis, allowing all three stylets to slowly sink into the created wound (Snodgrass 1946). The third or unpaired stylet is the epipharyngeal stylet. The epipharyngeal stylet is usually armed on its exposed anterior surface with a row of widely spaced nodules. These nodules may range from large tooth-like protuberances to hardly noticeable bumps depending on the species of flea (Snodgrass 1946). The inner posterior surface of the epipharyngeal stylet contains a pair of marginal flanges that form a canal or gutter which runs the entire length of the stylet. The tip or apical end of the epipharyngeal stylet is blunt (Snodgrass 1946).

When held in a natural position, both stylets of the maxillary lacinae sit side by side with their concave surfaces opposed, and partially enclose around the epipharyngeal stylet. The positioning of the three stylets, together with the "gutter" found on the epipharyngeal stylet, helps to create an epipharyngeo-lacinial tube, otherwise termed the food canal, which bloodmeals are drawn up and through by the sucking apparatus located within the head (Snodgrass 1946).

THE PICTORIAL KEY

After the discovery of the importance and role that fleas play in disease transmission in the late 1890s (Herms and James 1961), substantial efforts were made to understand the biology, habits, and anatomy of fleas, especially those with the capability to vector diseases to humans. With the abrupt end of Carl F. Baker's work on Siphonaptera in 1905, the death of Nathaniel C. Rothchild in 1923, accompanied later by the slowdown of Dr. Karl Jordan's work in 1938, so ended the efforts from some of the original and key researchers on the anatomy of fleas, who were invested in fleas for the flea's sake (Hubbard 1947). Also, the completion of research on medically important species stemming from the first recorded plague outbreaks in the United States, which occurred in San Francisco, CA in 1904, and the start of World War II, the study of flea anatomy slowed down around 1945, especially in the western region of the United States (Hubbard 1947). This slow-down caused many of the tools and materials created on the subject, which are still viable and useful, to become antiquated with time. Recent advances in photographic, computer, and wireless technology have made the possibility of photographing flea anatomy under high-powered microscopy readily accessible and plausible for experts or enthusiasts of the subject. With flea species being described in a time lacking such technology, the depth of research had its limits, causing many questions to remain on the nuances of flea anatomy, especially on a species level. This has left many flea taxonomic groups in need of revision, with some in complete disarray, such as the genus *Orchopeas*. This genus, which consists mainly of parasites found on tree squirrels, is one of the best representatives of the difficulties that one can find when identifying species within a given flea genera. With a high number of subspecies, and substantial variation in characteristics, some being very obscure, and most being picked from individual specimens rather than a series of individuals, many problems and confusions arise while attempting to identify species from this genus (Hubbard 1947).

Key morphological features used to identify flea species are represented in most references by either

written descriptions or line drawings, both of which are accurate, but can be very difficult for new students to the field of Siphonaptera to use effectively. Photographs of flea species are not readily available, and when accessible they are often taken of a specific species to demonstrate generalized anatomical structures for the entire order, in black and white, and printed onto the page of a book, limiting the user's access and ability to manipulate or magnify the image to obtain a better view. With the high variation of characteristics even among species or subspecies, it is common when first starting out to use anywhere from three to five different references to aid in identifying a species or even a single morphological feature. This is mainly due to the wide and unique terminology used to help relate the unusual anatomy of fleas to that of other insects, resulting in described body parts having multiple terms associated with them, which vary depending on the author or authority. One of the goals of this project was to create a new pictorial key, entitled *Pictorial Key to So me Common Fleas of Southern California*, which uses color pictures alongside short written descriptions to guide users accurately and efficiently to the proper identification of common flea species found on locally abundant backyard wildlife and pets in southern California.

The Orange County Vector and Mosquito Control District (OCVMCD) maintains flea and host mammal data spanning from 1997 to the present. Data from 1997 to 2016 was used to determine the most commonly found flea species on backyard wildlife and pets from within Orange County, CA. To obtain the data, OCMVCD examines host animals, usually postmortem, for the presence of ectoparasites (Table 1). Any fleas found on the host are collected, identified, sexed, and counted before either being tested for disease or being added to a reference collection. After reviewing the nearly twenty years of data, the twelve most common flea species from backyard wildlife were identified (Table 2).

Once the species list was determined, multiple references including such works as Hubbard (1947) and Lewis et al. (1988) were used to determine the best morphological features to use in the identification of the species. The main framework and inspiration for the structure and format of the new key was a series of "pictorial" keys created by the U.S. Department of Health and Human Services' Center for Disease Control and Prevention (CDC) (Fritz and Pratt 1947, Pratt 1947). Both keys are found in a collective reference covering multiple insects and other medically important animals entitled *Pictorial Keys: Ar thropods, Reptiles, Birds and Mammals of Public Health Significance* (USDHS CDC 1966). These keys use line drawings alongside short written descriptions to display different species and guide the user through the key anatomical features needed to identify them. Even though these two pieces were last revised in 1954, they give an excellent framework not only for the design of a pictorial key, but for how and what an individual should be looking at when identifying fleas.

With a framework for the structure of the key in place, the largest and most cumbersome task was to obtain mounted specimens of each species and capture photographs of the key structures and features used to identify them. Many of the more common specimens, such as *C. felis*, *Pulex irritans*, and *Echidnoghaga gallinacean* were obtained from the reference collection maintained at OCVMCD. To acquire photographs of mounted specimens of species not available in OCVMCD's reference collection, the author was invited to the Natural History Museum of Los Angeles County's Entomology Department and allowed access to their extensive flea collection. A collection of specimens was found and photographed, which was created by Clarence Hubbard, author of *Fleas of Western North Amer ica*, a reference used to compile descriptions found within the *Pictorial Key to Some Common Flea of Southern California*.

Table 1. List of host mammals examined for fleas in Orange County, CA.

Host Mammal (common name)	Species
Cat	*Felis catus*
Coyote	*Canis latrans*
Dog	*Canis lupus familiaris*
Mouse, house Mouse, California Deermouse, Northern Baja Mouse, Deer	*Mus musculus* *Peromyscus californicus* *Peromyscus fraterculus* *Peromyscus maniculatus*
Opossum	*Didelphis virginiana*
Rabbit	*Sylvilagus audubonii*
Raccoon	*Procyon lotor*
Rat, Norway Rat, roof	*Rattus norvegicus* *Rattus rattus*
Skunk	*Mephitis mephitis*
Squirrel, Eastern fox Squirrel, California ground	*Sciurus niger* *Spermophilus beecheyi*
Woodrats	*Neotoma bryanti* *Neotoma lepida* *Neotoma macrotis*

Table 2. Twelve common flea species found on backyard wildlife and pets in Orange County, CA.

Scientific Name	Common Name
Cediopsylla inaequalis interrupta	Common black rabbit flea
Ctenocephalides felis	Cat flea
Diamanus montanus	Ground squirrel flea
Echidnophaga gallinacea	Sticktight flea
Hoplopsyllus anomalus	N/A
Hoplopsyllus glacialis foxi	N/A
Leptopsylla segnis	Mouse flea
Nosopsyllus fasciatus	Northern rat flea
Orchopeas sexdentatus sexdentatus	N/A
Orchopeas howardii	Squirrel flea
Pulex irritans	Human/house flea
Xenopsylla cheopis	Oriental rat flea

Photographs for the key were obtained by using a Samsung® Galaxy Note5 smart phone, and a scope mounting device made by the company Phone Skope® (Beaver, UT), which allows a smart phone to be attached to the eyepiece of a microscope to acquire pictures. The mounting device consists of a case built for the specific model of smartphone the operator owns/uses, and a universal mount that attaches to the back of the case without blocking the camera lens, which is tightened around the eyepiece of the microscope. This device was chosen due to its low cost (compared to microscope cameras) and the mobility it offers, allowing the user to take their smart phone and mounting device to any location that has specimens and a compound microscope to acquire pictures.

Like the keys created by the CDC, the *Pictorial Key to Some Common Fleas of Southern California* starts by having the reader determine the presence or lack of genal and/or pronotal combs by grouping fleas into three main categories: 1) both genal and pronotal combs present; 2) only the pronotal comb present; and 3) no genal or pronotal comb present. Once it is determined that the flea fits into one of these three main categories, the key moves the user through branches of more specific anatomical features, all represented by written descriptions and pictures that are easily reached by tracing or tracking arrows provided to help guide the user from one key feature to another. If a user finds themselves at a feature that does not line up with the specimen they are working with, they can simply backtrack to the most recent correct feature by retracing the arrows provided and finding either the mistake that was made or branch that was bypassed. At the very end of each branch of the key, the user will be provided with an overall picture of both the female and male of the species, and the full scientific name. The largest difference in this newly created pictorial key compared to those used as its reference is that the new key has been designed as a digital file that can be downloaded onto a smart phone, tablet, laptop, or desktop computer. Working best with a touch screen, the key is easily manipulated by the user's hand, allowing one to magnify, and move to any portion of the key that they wish to view, or have been directed towards.

THE MOUNTING GUIDE

Once an individual begins to look at the anatomy of fleas and attempts to identify individual specimens, it will become clear quickly that many species of fleas will have to be cleared and mounted onto a microscope slide and viewed under a compound microscope to clearly see the features that are needed for proper identification. The act of clearing and mounting fleas is not new but can be a very obscure subject, with most of its references found in small chapters at the back of historical taxonomic books or keys, and very few if any writings or pieces dedicated to the topic alone. Clearing and mounting fleas can be a very challenging task, due to the specialized tools required for the work, the small size of the insect, and bodies which are laterally flattened, all of which will make working with these insects under a microscope feel alien at first. The second goal of this project was to create a guide to mounting fleas designed for public health professionals with little to no knowledge of fleas or how to work with them, and new students to the study of Siphonaptera or microscopy. The guide is entitled *How to Mount Your Flea: A Guide to the Preservation, Preparation, Clearing, and Mounting of Siphonaptera*, and combines steps/procedures from multiple historic and current references to create a guide that discuss the entire process from the start, preserving an unmounted specimen, all the way to the final steps of the process, sealing and labeling the slide. Users of the guide will find color pictures accompanying most of the key sections and steps, to make it easier to conceptualize the tasks that need to be performed.

To make the procedure easy to comprehend, the guide breaks the clearing and mounting process into multiple smaller sections/steps. The first section discusses the various materials and tools needed for the work, with pictures of each included. Homemade versions of some tools are also included in the first section. The second section unveils the proper way to preserve specimens prior to clearing and mounting. The third section, "The Four Steps to Clearing Your Flea," is the main portion of the guide and will require most of the user's time. Steps found in this section are: 1) potassium hydroxide (KOH) soak; 2) evacuating internal contents; 3) spreading and hardening the flea; and 4) cellosolve soak. Once users have cleared their flea, the guide will discuss the fourth section, mounting your flea, which describes how to mount the flea on a microscope slide. This section consists of two steps: 1) preparing the slide, and 2) mounting the flea. The final sections address how to seal and label the slide, thereby completing the process.

CONCLUSION AND FUTURE DIRECTIONS

Fleas, as the unique, remarkable, and unmistakable creatures that they are, have captivated the minds of many students and researchers over the years. Despite the ability of these tiny insects to mesmerize and captivate the minds of authorities like Carl F. Baker and Dr. Karl Jordan, the field and study of the anatomy of fleas slowed down over time. Due to the reduced pace of research coming out of the field in the mid-1940s, many of the tools and materials created on the subject have become antiquated over time. With many of the known flea species being described in a time lacking the technological advances we have today, many questions and avenues of research have also been left untouched. The key and mounting guide presented in this paper were produced to create easy and effective references for professionals and enthusiasts alike, and start eliminating the lag time affecting the advancement and creation of new taxonomical tools for the study of Siphonaptera through the use of modern technological advances. Both the *Pictorial Key to So me Common Fleas of Southern California* (2018) and *How to Mou nt Your Flea* are available for download at OMVCD's website, https://www.ocvector.org/flea-key-and-mounting-guide#body_file-ad90bd7e-8a94-4241-ad98-c8ac0b771eda.

Future plans for this project include the refinement and expansion of both documents that have been produced. With both the key and the mounting guide being in a digital format, any edits and additions that need

to be made to either can be performed with relative ease. The first plan is to add a new thirteenth species to the pictorial key, *Pulex simulans*. As OCMVCD has been focusing on mounting increasingly more specimens, there has been an adequate number of fleas that were previously determined or thought to be *P. irritans* or *P. spp.*, that are in fact *P. simulans*. Due to both *P. irritans* and *P. simulans* being morphologically identical externally, and the only differentiating feature being found on the aedeagus in the genitalia of males, it is common to mistake these species for one another if the time is not taken to clear and mount males that have been collected. The author has also been inspired to start performing histology techniques on future specimens in an attempt to delve into some of the untouched questions left in the field of Siphonaptera and produce more images that will allow for a better perspective and understanding of the truly unique anatomy known as the flea.

ACKNOWLEDGEMENTS

The authors would like to thank the entire staff of OCMVCD for all their work and support during the completion of this project, the Natural History Museum of Los Angeles County's Entomology Department for granting us access to their extensive flea collection and microscopes, and all the researchers that laid the ground work for the study of Siphonaptera.

LITERATURE CITED

Fritz, R., and H. Pratt. 1947. Fleas: pictorial key to species found on domestic rats in southern United States. U.S. Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center. Atlanta, GA.

Herms, W., and M. T. James. 1961. Medical entomolgy. Fifth edition. Macmillan Company, New York, NY.

Hubbard, C. A. 1947. Fleas of western North America. Iowa State College Press, Ames, IA.

Lewis, R. E., J. H. Lewis, and C. Maser. 1988. Fleas of the Pacific Northwest. Oregon State University Press, Corvallis, OR.

Orange County Mosquito and Vector Control District (OCMVCD). 2018. How to mount your flea. Garden Grove, CA.

Orange County Mosquito and Vector Control District (OCMVCD). 2018. Pictorial key to some common fleas of southern California. Garden Grove, CA.

Pratt, H. 1947. Fleas: pictorial key to some common species in the United States. U.S. Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center. Atlanta, GA.

Snodgrass, R. E. 1946. The skeletal anatomy of fleas (Siphonaptera). The Smithsonian Institution, Washington, D.C.

USDHS CDC (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention). 1966. Pictorial keys: arthropds, reptiles, birds and mammals of public health significance. U.S. Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center. Atlanta, GA.